A Polymorphism in the δ-Aminolevulinic Acid Dehydratase Gene May Modify the Pharmacokinetics and Toxicity of Lead

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Associations between the presence of a constitutional variant of δ-aminolevulinic acid dehydratase (ALAD-2) and lead concentrations in blood and bone, as well as between this allele and indices of kidney function, were investigated among 691 members of a construction trade union. The average blood lead level in this group was 7.78 µg/dl. No significant difference was observed in blood lead concentration in ALAD-2 carriers compared to those homozygous for the more common ALAD-1 allele (7.78 (± 3.62 µg Pb/dl vs. 7.73 (± 3.48 µg Pb/dl, respectively; $p = 0.73$). Bone lead was measured in a subset of 122 of the study subjects. Patella minus tibia lead concentrations for each individual averaged 3.35 ± 11.99 µg Pb/g bone mineral in ALAD-1 homozygotes and 8.62 ± 9.47 µg Pb/g bone mineral in ALAD-2 carriers ($p = 0.06$). Comparisons of blood urea nitrogen (BUN) and uric acid by genotype indicated elevated levels among ALAD-2 individuals ($p = 0.03$ and 0.07, respectively). In logistic regression models accounting for other variables potentially associated with BUN and uric acid levels, BUN was significantly associated with blood lead levels ($p = 0.01$). Associations of BUN and uric acid levels with ALAD-2 were of borderline statistical significance in these models ($p = 0.06$ and 0.07). Taken together, these results suggest that the ALAD-2 genotype may influence the pharmacokinetic distribution and chronic renal toxicity of lead, perhaps due to differential binding of lead to the variant protein. *Key words*: aminolevulinic acid dehydratase, biomarkers, bone lead, kidney function, lead, susceptibility. *Environ Health Perspect* 103:248–253 (1995)

Exposure to lead constitutes a serious occupational and environmental health problem (1–3). At high exposure levels, lead has been demonstrated to cause encephalopathy (4,5), kidney damage (6–9), anemia (2), and toxicity to the reproductive system (10,11). Lead exposure may also lead to elevated rates of hypertension (12–14). At lower doses, lead has been associated with alterations in cognitive development in children (14–19). The nature and degree of the health risks posed by exposures to low levels of lead are a matter of debate but, to date, unequivocally safe exposure levels have not been identified (1).

The health risks associated with low-level exposures to lead have important implications with respect to its regulation. Standards and health-based guidelines limiting occupational and environmental exposures to lead have become more stringent over the past decade and are now thought to protect against major adverse outcomes. However, as is the case with most toxins, the low-dose risks posed by lead are likely to be determined, in part, by individual determinants of susceptibility. A failure to adequately consider these factors in the establishment of regulatory standards may result in the unacknowledged imposition of substantial risks on some members of society.

Recent work has implicated a genetic trait as a possible determinant of susceptibility to higher blood lead levels. A relatively common variant of the second enzyme in heme biosynthesis, δ-aminolevulinic acid dehydratase (ALAD) has been reported to be associated with susceptibility to higher blood lead concentrations among children and workers (20–23). The association of this variant, designated ALAD-2, with elevated blood lead levels was observed in populations exposed to lead at relatively high levels; blood lead concentrations in the three reported studies investigating this issue were generally in excess of 10 µg Pb/dl (20–23).

The ALAD-2 allele is found in approximately 11–20% of whites (24,25) and has been postulated to act as a modifier of the pharmacokinetics and toxicity of lead (20). Although the precise mechanism involved in the biological action of this genotype is unknown, it may enhance the uptake, decrease the elimination, and/or modify the tissue distribution of lead in the body perhaps due to an increased affinity of the variant protein for lead. This possibility follows from the observation that ALAD is strongly inhibited by lead, which is thought to exert this effect by displacing zinc from the enzyme (26,27). Depending on the target organ, such pharmacokinetic effects could either enhance or reduce the ultimate toxicity of lead.

To further investigate possible alterations in lead kinetics associated with ALAD genotype, we have studied members of a construction trade union. In this population, lead exposure may occur during job-related activities such as the demolition and renovation of old buildings containing lead paint and work using lead-based solder, for example. This group was expected to have relatively modest overall exposures to lead, allowing for the potential impacts of ALAD-2 on lead body burdens to be considered at levels more representative of exposures occurring among the population at large. We determined ALAD genotype, blood lead concentrations, and bone lead concentrations in both the patella and tibia. Relationships between genotype and blood pressure and kidney function, endpoints that have previously been demonstrated to be adversely affected by lead, were also investigated.

Methods

Volunteers were recruited at the 1991 International Brotherhood of Carpenters and Joiners convention in Atlantic City, New Jersey. Participants completed a detailed questionnaire covering demographic, lifestyle, and occupational histories and were screened for various medical conditions including hypertension. Blood samples were voluntarily donated for determination of blood lead levels and ALAD genotype. No exclusion criteria were applied. This research was approved by the Human Subjects and Research Committee of the Brigham and Womens Hospital.

Lead determinations were made by

Hoffman-LaRoche Laboratories using anodic stripping voltametry (ESA model 3010). Eight percent of the samples consisted of references obtained from the New York Department of Health and were analyzed blind. The coefficients of variation for blood lead determinations from this laboratory have been estimated as 10, 8, and 5–6% at blood lead concentrations of 1–10, 10–30, and 30–60 µg/dl, respectively.

For genotyping we used a polymerase chain reaction (PCR)-based technique that allows ALAD-1 and ALAD-2 alleles to be rapidly differentiated from small samples of whole blood (28). Briefly, amplifications were performed essentially as described (21) on 0.5 µl of whole blood using two sets of primers specific for a portion of the ALAD gene. Primer sequences for the initial round of amplification were (5'–AGACAGACATTAGCTCAGTA-3') and (5'-GGCAAAGAACACGTCCAT-TC-3'), which generate a 916 base pair fragment. To increase the amplification yield, which was inconsistent from this first amplification step when using whole blood, a second round of amplification was performed using a nested pair of primers (5'-CAGAGCTGTTCCACAGTGGA-3') and (5'-CCAGCACAATGTGGGAGT-GA-3') which generate a 887 base pair fragment. Amplified DNA was then restricted using *MSP*I and electrophoresed through 2.0% agarose. ALAD alleles are differentiated based on the existence of a *MSP*I endonuclease restriction site specific to the ALAD-2-derived PCR fragment, which yields a diagnostic restriction band. Heterozygotes exhibit both the ALAD-1 and ALAD-2 fragments and can thus be differentiated from homozygotes of either type. We performed PCR reactions in duplicate with blank controls included in each set.

Patella and tibia bone lead determinations were made on a subset of those participating using a prototype ABIOMED K-XFR instrument as described previously (29). Briefly, this instrument uses a ^{109}Cd γ-ray source to provoke the emission of fluorescent photons from target tissues. By estimating counts derived from both lead and bone matrix, a measurement in units of micrograms lead per gram bone mineral is derived. The uncertainty in the measurement increases with thicker overlying tissue and lower bone density. For this data set estimates of measurement uncertainty, based on the strength of the returning signal, were obtained for each individual, yielding an overall uncertainty of 7.0 ± 1.4 and 8.0 ± 1.6 µg/g for the tibia and patella, respectively.

At very low bone lead concentrations, the K-XFR instrument may generate negative point estimates of bone lead, especially if the associated measurement uncertainty

is large. These negative values are, however, informative as they become more likely at lower bone lead concentrations. Thus, negative values were retained in the analyses. A complete discussion of this approach is reviewed in Hu et al. (30).

We analyzed relationships between genotypic status and blood and bone lead concentrations as well as blood pressure and three markers of kidney function using Student's *t*-test, multivariate logistic regression, and Wilcoxon-rank sum methods. Because lead partitioning to trabecular bone (here represented by the patella) is quite distinct from partitioning to cortical bone (here represented by the tibia) (31,32), we also examined the influence of ALAD status on the difference between tibia and patella bone lead concentrations (i.e., the cortical-trabecular bone lead differential).

Results

Demographic characteristics of the study population are presented in Table 1. Volunteers were predominantly white males with an average age of 48 years. Blood lead values and ALAD genotypes were obtained for 691 individuals. Bone lead determinations were obtained on a subset of 131 individuals from this larger group; time constraints did not allow for the testing of additional volunteers. Nine people either did not adequately complete the questionnaire (n = 4) or were not genotyped because blood samples were not available (n = 5), leaving a total of 122 individuals for analysis.

Figure 1 presents a representative ethidium-bromide stained gel displaying the distinguishing restriction fragments used to identify ALAD genotypes. A total of 594 ALAD 1-1 homozygotes (85.96%), 95 ALAD 1-2 heterozygotes (13.74%) and 2 ALAD 2-2 homozygotes (0.29%) were identified. In further analyses the ALAD 1-2 heterozygotes and 2-2 homozygotes have been combined to generate a single category of individuals carrying at least one ALAD-2 allele. Although ALAD gene
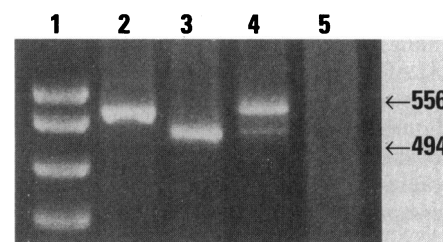
Figure 1. Polymerase chain reaction-based determination of ALAD genotype. ALAD 1-1 homozygotes are characterized by a single 556 base pair *MSP*I fragment (e.g., lane 2), ALAD 2-2 homozygotes by a single 494 base pair fragment (e.g., lane 3), and heterozygotes contain both (e.g., lane 4). Lane 1 contains molecular weight standards (*MSP*I digested pBR322), and lane 5 is the control.

dosage effects on lead pharmacokinetics may be possible, the limited number of ALAD 2-2 homozygotes identified in this population preclude any meaningful analysis of this possibility.

Blood lead values in this population were relatively low, as expected, averaging 7.78 (± 3.60) µg Pb/dl. Average blood lead concentrations were essentially identical between ALAD genotypes (7.78 ± 3.62 versus 7.73 ± 3.48 µg Pb/dl for ALAD-1 and ALAD-2, respectively; p = 0.73) (Table 2). Plots of the cumulative frequency distribution of blood lead values by genotype also revealed no apparent differences across the blood lead concentration range observed in this population (data not shown).

Overall, bone lead concentrations were relatively low in this population (9.43 ± 8.93 and 13.82 ± 11.77 µg Pb/g bone mineral for tibia and patella, respectively) (Table 2). Both tibia and patella bone lead concentrations were significantly correlated with age (p = 0.001).

Age-adjusted bone lead values, using residuals of least-square regression, were used to compare bone lead concentrations between genotypes quantitatively. Age-adjusted tibia values were somewhat higher and patella values were lower in the ALAD-1 group. Overall, average age-adjusted tibia

Table 1. Demographic characteristics of the study population[a]

Characteristic	Total	ALAD-1/1[b]	ALAD-1/2 and -2/2[c]
Complete cohort			
N	691	594	97
Age	48.1 ± 8.8	48.0 ± 8.8	49.2 ± 8.9
Alcohol (drinks/day)	0.2 ± 0.7	0.2 ± 0.7	0.1 ± 0.6
Smoking (cigarettes/day)	17.1 ± 15.6	17.3 ± 15.7	16.3 ± 14.9
Subgroup with bone lead determinations			
N	122	101	21
Age	48.3 ± 9.9	48.2 ± 10.1	49.2 ± 8.9
Alcohol (drinks/day)	0.9 ± 1.4	1.0 ± 1.4	0.6 ± 1.2
Smoking (cigarettes/day)	16.0 ± 15.4	15.8 ± 15.6	16.9 ± 14.5

[a]Values are means ± SD.
[b]ALAD 1/1, homozygous wild type.
[c]ALAD 1/2 and 2/2, heterozygous and homozygous variant for ALAD-2 allele.

lead concentrations were 7.50 (± 7.70) µg Pb/g bone mineral among the combined ALAD-2 heterozygote/homozygote group versus 9.87 (± 8.16) µg/g among those homozygous for ALAD-1 (*p* = 0.29). In contrast, ALAD-2 age-adjusted patella lead values were higher: 15.98 (± 6.47) µg/g compared to 13.37 (± 12.57) µg/g among ALAD-1 homozygotes (*p* = 0.10).

Possible genotypic effects on the relative distribution of lead between the tibia and patella (cortical versus trabecular bone types) was investigated by subtracting the measured lead concentrations in patella bone from those observed in tibia bone for each individual. The resulting population mean values were 3.35 (± 11.99) and 8.62 (± 9.47) µg Pb/g bone mineral, for ALAD-1 and ALAD-2, respectively. This difference was of borderline statistical significance (*p* = 0.06).

Population mean systolic and diastolic blood pressures were not significantly elevated among ALAD-2 individuals (Table 3). Comparisons of the number of individuals expressing systolic blood pressure above 140 and diastolic blood pressure above 90 showed no significant association with ALAD genotype (not shown).

Blood urea nitrogen (BUN), uric acid, and creatinine were all elevated among ALAD-2 individuals (Table 3). The difference in BUN values between genotypes was statistically significant (*p* = 0.03), while those for uric acid and creatinine were of borderline significance (*p* = 0.07 and *p* = 0.10, respectively).

Potential relationships between BUN, uric acid, and creatinine levels with ALAD genotype were investigated further using logistic regression models incorporating the additional variables of age, blood lead concentration, and alcohol consumption, factors potentially related to kidney function. Table 4 presents parameter estimates, standard errors, and *p*-values for the various models. Initially, all variables (including genotypic status) were included as independent variables. Variables were dropped until only those with *p*-values of less than 0.20 remained. Values for these final models are presented in Table 4.

Interestingly, blood lead concentrations alone significantly predicted BUN outcomes even at these low lead levels, with ALAD genotypic status also being of borderline statistical significance as an explanatory variable (*p* = 0.06). For all logistic models, ALAD status yielded the highest parameter estimates and, in the case of uric acid, was also of near statistical significance as an explanatory variable (*p* = 0.07). In this latter case, ALAD genotype was the only variable that approached statistical significance.

Discussion

Inhibition of ALAD enzymatic activity is a sensitive indicator of exposure to lead, exhibiting a dose–response relationship over a broad range of blood lead concentrations (*26*). This inhibition may be due to the binding of lead to the enzyme, with displacement of the normal metallic cofactor, zinc (*27*). Astrin et al. (*20*) previously reported a statistically significant overrepresentation of the ALAD-2 isozyme among individuals with blood lead concentrations in excess of 30 µg/dl compared to those with levels below 20 µg/dl (*20*). A follow-up study by Wetmur et al. (*22*) confirmed this observation. In a smaller study of 202 occupationally exposed workers, individuals carrying ALAD-2 also expressed higher blood lead levels than ALAD 1-1 homozygotes (*23*). Based on these observations, it has been suggested that the ALAD-2 form of the enzyme may exhibit a greater affinity for lead, increasing the overall uptake and retention of lead in the body. Increased

susceptibility to the toxic effects of lead have also been postulated, although no investigations to date have explicitly addressed this issue.

Previous studies focused on populations with relatively high exposures to lead; although large cohorts were involved, few individuals had blood lead levels below 10 µg/dl. Thus, these investigations did not allow for a careful consideration of potential genotypic associations with lower blood lead levels. Additionally, interpretation of the Astrin and Wetmur studies is complicated by the fact that enrollment into these investigations was based, in large part, on initial clinical evaluations of elevated erythrocyte protoporphyrin (FEP) levels (*20,22*). This introduces a potentially serious selection bias in the study design. For example, if ALAD-2 reduced sensitivity to lead-induced FEP elevation, higher blood lead levels would be required for this genotype to be "captured" by the study, leading to a spurious association of geno-

Table 2. Blood and bone lead concentrations by δ-aminolevulinic acid dehydratase (ALAD) genotype[a]

		Genotype		
	Total population	ALAD-1	ALAD-1/2 and -2/2	*p*[b]
All carpenters				
N	688	592	96[c]	
Blood lead level (µg/dl)	7.8 ± 3.6	7.8 ± 3.6	7.7 ± 3.5	0.89[d]
Bone lead subcohort				
N	122	101	21	
Patella	13.7 ± 13.4	13.1 ± 14.1	16.4 ± 9.2	0.18
Tibia	9.4 ± 8.9	9.8 ± 9.1	7.8 ± 8.1	0.36
Adjusted patella	13.8 ± 11.8	13.4 ± 12.6	16.0 ± 6.5	0.17
Adjusted tibia	9.5 ± 8.1	9.9 ± 8.2	7.5 ± 7.7	0.22
Blood lead level (µg/dl)	8.0 ± 3.9	8.0 ± 4.1	8.2 ± 3.2	0.85
Patella-tibia difference	4.3 ± 11.7	3.4 ± 12.0	8.6 ± 9.5	0.06[c]

[a]Values are means ± SD.
[b]Wilcoxon and rank sum analysis, Kruskal-Wallis chi-square approximation, unless noted otherwise.
[c]ALAD 1-2, *N* = 94; ALAD 2-2, *N* = 2.
[d]*t*-test.

Table 3. Markers of kidney function and blood pressure levels by genotype[a]

	Genotype		
Marker	ALAD-1	ALAD-1/2 and -2/2	*p*
Blood urea nitrogen (mg/dl)	18.5 ± 4.6	19.6 ± 4.5	0.03
Uric acid (mg/dl)	6.4 ± 1.4	6.7 ± 1.6	0.07
Creatinine (mg/dl)	1.2 ± 0.2	1.3 ± 0.2	0.11
Diastolic blood pressure	84.1 ± 9.1	85.5 ± 9.2	0.16
Systolic blood pressure	133.5 ± 17.5	134.9 ± 15.0	0.47

ALAD, δ-aminolevulinic acid dehydratase.
[a]Values are means ± SD.

Table 4. Summary of logistic regression model for markers of kidney function

	BUN		Uric acid		Creatinine	
Model variables	Estimate	*p*	Estimate	*p*	Estimate	*p*
ALAD status[a]	0.91	0.06	0.28	0.09	0.03	0.16
Blood lead level (µg/dl)	0.13	0.005	0.002	0.91	0.001	0.50
Age (years)	0.12	0.0001	0.002	0.77	0.002	0.009
Alcohol consumption (drinks/day)	-0.04	0.87	0.13	0.12	0.003	0.77

Abbreviations: ALAD, δ-aminolevulinic acid dehydratase; BUN, blood urea nitrogen.
[a]ALAD status: ALAD 1-1 or ALAD 1-2, 2-2.

type with blood lead levels. If the converse were true, a bias toward the null hypothesis of no genotype effect would occur.

The results reported here avoid this potential selection bias by screening individuals directly for blood and bone lead concentrations. We have also focused on a population likely to have had modest exposure to lead, a situation more representative of current overall population exposures. This allows the relationship between genotype and blood lead levels at low doses to be more fully investigated. Finally, we have initiated investigations into the possible association of this genotype with bone lead concentrations and certain indicators of lead toxicity.

Overall, blood lead levels in the population of union members studied here averaged 7.78 (± 3.60) µg Pb/dl. These results indicate that current lead exposures among this group are generally low. It is important to note, however, that the union members studied here may not be representative of the overall union membership, which may be composed of a higher percentage of individuals at risk for on-the-job lead exposure. Many of our subjects were full-time union representatives who had not been actively working at construction sites for several years (*29*).

Our results indicate that the ALAD-2 genotype is not a significant determinant of blood lead concentrations among individuals exposed at relatively low levels. Blood lead concentrations were essentially identical between the ALAD genotypes (Table 2) in this population. This suggests that the association observed at higher blood lead levels may not pertain to the low-level exposure situation. The higher level effect could be mechanistically dependent on the kinetics of lead uptake and saturation of binding to other blood proteins. For example, some forms of hemoglobin bind lead with great affinity (*33,34*). The effect of the variant ALAD protein may therefore be significant only after saturation of such hemoglobin-associated lead-binding sites and could thus be evidenced only transiently after exposure to high levels of lead. A recent study of occupationally exposed Koreans, screened directly on the basis of blood lead levels, also supports this possiblity: ALAD-2 was overrepresented only among individuals with blood lead levels greater than 40 µg/dl (*35*).

Although blood lead concentrations have been used extensively to determine lead uptake, the utility of this measurement is limited to relatively recent exposures. Because the half-life of lead in the blood is 28–36 days, blood lead concentrations only provide a reliable measure of exposures that have occurred over a period of a few weeks (*36*). Estimates of longer-

term lead exposures are better provided by determinations of bone-lead concentrations, which can be made using X-ray fluorescence techniques developed over the past decade (*37*).

Even though the overall pharmacokinetic distribution of lead in the body is complex, it is clear that bone tissue constitutes the major long-term storage depot for this metal (*38*). The concentration of lead in bone thus provides an integrated estimate of exposures that have occurred over a much longer period and are of toxicological concern, as these stores can be mobilized, leading to continued systemic exposures long after environmental exposures have ceased (*38*). Bone serves as a long-lived sink for up to 95% of total body burdens of lead in adults (*1*).

Differences in lead accumulation in various bone types has been noted. Patella lead concentration values often differ from those observed in the tibia (*30–32*). The cortical bone of the tibia represents a long-term storage depot which would be expected to exhibit a half-life for lead in excess of a decade. In contrast, the more dynamic trabecular bone of the patella exhibits a shorter half-life. The precise mechanism accounting for this difference is not known, but is likely to be a function of differences in blood perfusion, rates of bone turnover, and specific structural and compositional aspects of the bone matrix.

The cumulative nature of bone lead concentrations and the expected differences in partitioning between patella and tibia bone suggest that lead concentrations in these two depots could provide a sensitive indicator of genotype-related differences in the overall pharmacokinetics of lead. Lead concentrations in these compartments were therefore determined for a subset of those participating in this study.

The observed difference between patella and tibia bone lead concentrations between genotypes (Table 2) suggests that ALAD status may modify the way in which lead partitions between these bone depots. Cortical bone concentrations will better reflect earlier periods of potentially higher lead exposure in this population. The noted differences between cortical and trabecular bone lead levels would be consistent with a reduction in lead uptake into long-term cortical bone storage sites, perhaps via enhanced binding to ALAD-2 peripherally during periods of elevated occupational exposure to lead. That is, the variant ALAD-2 protein may effectively increase the blood and soft tissue (e.g., spleen and kidney) compartment half-lives of lead, decreasing partitioning to the cortical bone compartment. This would be expected to increase the cumulative exposure to trabecular bone such as the patella.

Such a change in the distribution of lead could also influence the partitioning of lead to other tissues as well, potentially leading to complex relationships between genotype and toxicity. For example, ALAD-2 may serve to sequester lead in a form that is inaccessible to certain organs such as the central nervous system. That this may be the case is suggested by a recent follow-up study of 72 adolescents where all 5 individuals carrying ALAD-2 performed better on a battery of neuropsychological tests compared to those homozygous for ALAD-1 (*39*). In contrast, decreased partitioning to long-term bone storage could simultaneously increase the cumulative delivery of lead to other organ systems where the bound lead might be more toxicologically available, perhaps due to cellular processing such as might occur during tubular transport in the kidney. This could well exacerbate lead toxicity at some sites.

Indeed, our results do suggest that low-level blood lead concentrations may be associated with subclinical kidney effects (as evidenced by an increase in BUN values) and that ALAD-2 may contribute to this. In logistic regression models with BUN as the dependent variable and age, ALAD status, and blood lead concentrations as the independent variables, blood lead concentration was significantly associated with BUN values ($p = 0.01$). Lead-induced renal toxicity has been well established in humans at relatively high blood lead concentrations (*6–9,40–43*) and has been suggested to occur at more modest levels as well (*44–46*). Manifestations of acute lead-induced kidney toxicity primarily involve damage to the kidney tubules with appearance of nuclear inclusion bodies, overt dysfunction, decreased uric acid excretion, and cytomegaly of the epithelial cells of the proximal tubules (*38*). Although many of these effects are considered reversible, prolonged or repetitive exposure to lead may result in cumulative kidney damage with potential incremental loss function via progressive interstitial fibrosis (*6*).

In workers with average blood lead levels in excess of 80 µg/dl, creatinine clearance has been reported to be reduced (*41*). BUN and serum uric acid levels were also found to be elevated significantly in workers with blood lead concentrations of 72 (± 17) ug Pb/dl (*40*). In another study, subclinical kidney damage was detected through an association between blood lead and *N*-acetyl-D-glucosaminidase, a sensitive lysosomal marker of tubular cell toxicity. Blood urea nitrogen, uric acid, and creatinine levels were, however, all within normal ranges in this study (*42*). A weak but statistically significant association between blood

lead levels and BUN and serum creatinine among 209 Japanese workers, only 5 of whom exceeded 60 µg Pb/dl, has also been reported (44), suggesting that lead-associated effects on these parameters may occur at more modest blood lead levels. Finally, two recent epidemiological studies have also reported a dose-response relationship between relatively low levels of lead in blood (5–25 µg/dl) and decreased creatinine clearance (45,46).

Current blood lead levels may not provide an adequate dosimeter with respect to kidney toxicity. Even short-term, high-level lead exposures could hypothetically contribute to clinical or subclinical effects many years after exposure, even if the effects appear to have reversed. Repair of toxicant-induced kidney damage is unlikely to perfectly restore the damaged tissues and may thus lead to a permanent decrement in the functional reserve capacity. Incremental loss of kidney function resulting via other mechanisms such as the normal aging process and exposures to other renal toxins could lead to functional insufficiencies that might otherwise not have developed. Such a mechanism may be involved in the lead-associated BUN elevation observed here, in effect preserving a rank order of effects attributable to earlier higher-level lead exposures. Alternatively, these results may be indicative of low-level lead effects on kidney function. Such effects have been suggested in other recent studies (42–45).
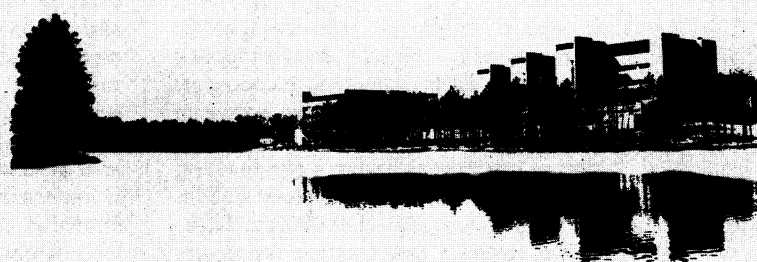
Our results also suggest a potential ALAD-2 relationship with lead-associated renal effects. Elevated levels of BUN, uric acid, and creatinine were all observed among ALAD-2 versus ALAD-1 individuals in this population. Considered in isolation, the association with BUN was statistically significant ($p = 0.03$), but when modeled, including other potentially significant parameters, the associations between genotype and BUN and uric acid remained of borderline statistical significance ($p = 0.06$ and $p = 0.07$, respectively). Although ALAD-2 status could potentially influence kidney function via a mechanism independent of lead, these results are consistent with the potential ALAD effects on lead distribution discussed earlier. A decrease in the relative distribution of lead to long-term bone stores, even if restricted to intermittent periods of high-level lead exposure, where ALAD-2–blood lead associations have been observed, would be likely to increase the cumulative amount of lead accessible to the kidney, potentially exacerbating toxicity at this target organ. As ALAD-2 is predominantly associated with blood lead concentrations in excess of 30 µg/dl, such kidney effects would be anticipated to be more pronounced among those experiencing higher-level exposures.

In conclusion, the observed differences in bone lead concentrations between the tibia and patella are suggestive of potential ALAD-2-associated pharmacokinetic effects, perhaps due to a greater affinity of the variant protein for lead. Such a difference may be of toxicological importance; depending on the target organ ALAD-2 could protect against ultimate toxicity (e.g., central nervous system effects) or enhance toxicity (e.g., renal effects) due to altered partitioning and availability of complexed lead. Our results support this possibility, suggesting that subclinical lead-associated kidney dysfunction is associated with relatively low current blood lead concentrations and that the ALAD-2 genotype may be an additional modifier of this effect. Further follow-up of these issues among populations exposed to lead at higher levels, where ALAD-2 effects on blood lead levels have been noted, are needed to confirm these observations.

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